ISSN 1330-9862
(FTB-2603)

original scientific paper

Demethylation of *N*,*N*-Dimethylbenzenamine and *N*,*N*,3-Trimethylbenzenamine by Whole Cells of *Aspergillus terreus*

Leandro Piovan[1], *Edna Kagohara*[1], *Juliana Alves Vale*[1], *João Valdir Comasseto*[1], *Iracema Helena Schoenlein-Crusius*[2], *Leandro Helgueira Andrade*[1] *and André Luiz Meleiro Porto*[1,3]*

[1]Institute of Chemistry of São Paulo, University of São Paulo, Av. Prof. Lineu Prestes, 748, CEP 05508-900, São Paulo-SP, Brazil

[2]Institute of Botany of São Paulo, Section of Mycology and Lichenology, Av. Miguel Stéfano, 3687, CEP 01061-970, São Paulo-SP, Brazil

[3]Institute of Chemistry of São Carlos, University of São Paulo, Av. Trabalhador São-Carlense, 400, CEP 13566-590, São Carlos-SP, Brazil

Received: June 28, 2010
Accepted: October 1, 2010

Summary

N,*N*-dimethylbenzenamine and *N*,*N*,3-trimethylbenzenamine were *N*-demethylated through enzymatic reactions mediated by whole cells of *Aspergillus terreus* strains SSP 1498, URM 3371 and URM 3571. The respective products, *N*-methylbenzenamine and *N*,3-dimethylbenzenamine, were obtained in high conversions under both neutral and basic conditions. The oxidative *N*-demethylation of tertiary aromatic amines by *A. terreus* was not enhanced by the presence of the oxidant *tert*-butylperoxide, although further demethylation of *N*,*N*-dimethylbenzenamine to aniline was observed. The strains of the investigated *A. terreus* were unable to perform the dealkylation of *N*,*N*-diethylbenzenamine.

Key words: Aspergillus terreus, *N*-demethylation, tertiary aromatic amines, biotransformation

Introduction

Tertiary *N*-alkyl groups are present in numerous natural products, and especially in alkaloids and their derivatives. The *N*-methyl group is a characteristic moiety associated with many naturally occurring alkaloids of biological importance, as the analgesic opiates morphine, codeine or nicotine (*1*,*2*). Whilst it is often required to eliminate the *N*-methyl groups of tertiary amines, the process is difficult when the substrate presents multiple functionalities and/or stereogenic centres. *N*-demethylation may be mediated by a number of chemical methods including reaction with BrCN (the Von Braun reaction) (*3*), *N*-iodosuccinimide (*4*), solid reagents such as silica gel (*5*), ruthenium catalyst (*6*), iodine and calcium oxide (*7*), iodozylbenzene (*8*), 1-chloroformate (*9*) and iron(II) (*10*), and also through photochemical procedures (*11*). In nature, oxidative enzymes mediate *N*-demethylation reactions *via* a mechanism that typically involves electron transfer catalysed by cytochrome P450 (*12–15*). However, it is possible that *N*-demethylation occurs through an alternative pathway mediated by monooxygenases. Thus, it is known that monooxygenases can catalyse the oxidation of *N*-tertiary amines to produce the corresponding *N*-oxides (*16*). Additionally, *N*-oxide intermediates can be biotransformed into *N*-demethylated products by various enzymes. Moreover, deoxygenative demethylation

*Corresponding author; Phone: ++55 16 3373 8103; Fax: ++55 16 3373 9952; E-mail: almporto@iqsc.usp.br

reactions of *N*-oxide intermediates occur *via* carbenium--iminium ions (Mannich-type intermediates) that can be formed by the decomposition of an *N*-oxide (*17*,*18*).

We have recently utilised whole cells of the fungus *Aspergillus terreus* to bring about the deracemisation of alcohols (*19*), the biotransformation of Mannich bases (*20*) and selective oxidation of organoseleno alcohols (*21*). In the present study, the dealkylation reactions of the *N*,*N*--dimethylbenzamine (**1**) and *N*,*N*,3-trimethylbenzenamine (**2**), mediated by *A. terreus* as biocatalyst (Scheme 1), have been investigated both in the presence and absence of the oxidant *tert*-butylperoxide (*6*). The reactions were analysed by GC-MS with electron impact (EI) ionisation in order to determine the formed products and with electron spray ionisation (ESI) in an attempt to detect possible intermediates.

Material and Methods

General methods

Sterile material was used to perform the experiments and the microorganisms were manipulated in a Veco® (Campinas, SP, Brazil) laminar flow cabinet. Incubations of biocatalyst with substrate were carried out on Technal TE-421 (Piracicaba, SP, Brazil) or Superohm G-25 (Piracicaba) orbital shakers. Amines **1** and **2** and *N*-methylbenzenamine (**1a**) were obtained commercially, whilst *N*,3--dimethylbenzenamine (**2a**) was identified by GC-MS analysis. The IUPAC names of all compounds mentioned were verified using ChemDraw Ultra software (v. 8.0; CambridgeSoft, Cambridge, MA, USA).

Thin layer chromatography (TLC) using aluminium--backed silica gel 60 F_{254} layers (Merck, Jacarepaguá, RJ, Brazil) eluted with hexane and ethyl acetate was employed to monitor the biocatalysed reactions. Analytes were visualised by spraying with *p*-anisaldehyde/sulphuric acid reagent or with vanillin, followed by heating at approx. 120 °C for a maximum of 1 min. GC-MS analyses were carried out on a Shimadzu P5050A (Shimadzu, Kyoto, Japan) GC-MS instrument fitted with a DB-5 (J&W Scientific, Folsom, CA, USA) fused silica capillary column (30 m×0.25 mm i.d; film thickness 0.25 μm). The chromatographic conditions were: oven temperature programmed from 50 to 230 °C at a rate of 10 °C/min, injector temperature set at 230 °C, split ratio fixed at 1:20, carrier gas (helium) pressure set at 100 kPa, and MS interface temperature maintained at 250 °C.

Sources of microorganisms

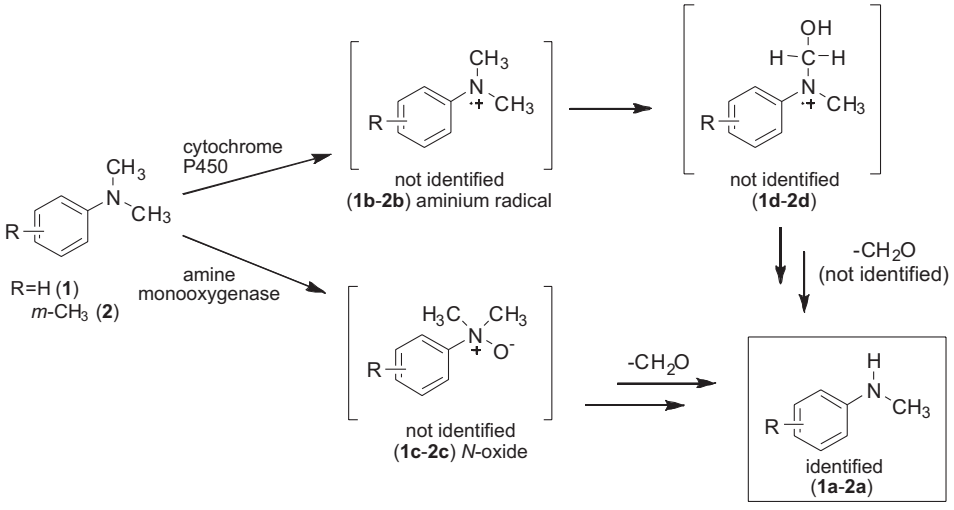
The culture strains *A. terreus* URM 3371 (isolated in 1993 from rhizosphere soil of *Vernomia herbaceae* in São Paulo-SP, Brazil) and *A. terreus* URM 3571 (isolated in 1996 from a water reservoir in northeastern Brazil) were obtained from the culture collection of the Department of Mycology of the Federal University of Pernambuco, Recife-PE, Brazil. *A. terreus* SSP 1498 is a historic strain from the culture collection of the São Paulo Botanical Institute and was isolated in the USA (probably from the soil) by Dr Enrique Duprat and freeze-dried in 1943 at the Northern Regional Research Laboratories, Peoria, IL, USA.

Growth conditions for microorganisms

Cells of *A. terreus* were grown in 2-litre Erlenmeyer flasks containing 1 L of Oxoid (São Paulo, SP, Brazil) malt extract medium (20 g/L) and maintained at 32 °C on an orbital shaker (160 rpm) for 96 h. The cells were harvested by filtration under vacuum. Following filtration, a batch of cells of the strain URM 3571 was stored in a closed flask for 1 year in a refrigerator maintained at 4 °C.

Procedure for small-scale biocatalytic reactions

An aliquot (5, 10 or 20 μL) of the tertiary amine substrate was added to a 125-mL Erlenmeyer flask containing wet cells (5 g) of *A. terreus* strains SSP 1498, URM 3371 or URM 3571 suspended in either 50 mL of 1 M phosphate buffer (Na_2HPO_4/KH_2PO_4; pH=7) or 50 mL of 1 M borate buffer (H_3BO_3/KCl; pH=9). In some experiments, 20 μL of *tert*-butylperoxide were added to the incubation mixture. Incubations were carried out at 32 °C on an orbital shaker (160 rpm) for the time periods



Scheme 1. Possible pathways for the biotransformation of tertiary aromatic amines *N*,*N*-dimethylbenzenamine (**1**) and *N*,*N*,3-trimethylbenzenamine (**2**) by whole cells of *Aspergillus terreus*

shown in Tables 1–3. In one set of experiments, cells of *A. terreus* URM 3571 were stored at 4 °C for 1 year prior to being used in the biocatalytic reactions as outlined above.

Analysis of the course of biocatalysed reactions

The progress of each reaction was monitored by collecting 2-mL samples of the mixture at various times (Tables 1–3). Samples were extracted by stirring with ethyl acetate (1 mL), and aliquots (3 μL) of the organic phases were analysed by capillary GC-MS. In addition, TLC analyses were also employed to monitor the biocatalysed reactions by using aluminium-backed silica gel 60 F_{254} layers eluted with hexane and ethyl acetate (8:2). The structures of the demethylated products **1a** and **2a** of the biocatalysed reactions were identified and confirmed by reference to the Shimadzu Class-500/Wiley mass spectral database (Wiley, Hoboken, NJ, USA): *N*,*N*-dimethylbenzenamine (**1**): MS (EI) *m/z* (relative intensity in %): 120 [M^+] (100), 121 [M^++1] (78), 77 (30); *N*-methylbenzenamine (**1a**): MS (EI) *m/z* (relative intensity in %): 106 [M^+] (100), 107 [M^++1] (83), 77 (36); *N*,*N*,3-trimethylbenzenamine (**2**): MS (EI) *m/z* (relative intensity in %): 134 [M^+] (100), 135 [M^++1] (71), 91 (24); and *N*,3-dimethylbenzenamine (**2a**): MS (EI) *m/z* (relative intensity in %): 120 [M^+] (100), 121 [M^++1] (91), 107 (34).

Procedure for the extraction of the products of biocatalytic reactions

An aliquot (100 μL) of tertiary amine substrate **1** or **2** was added to a 500-mL Erlenmeyer flask containing wet cells (15 g) of *A. terreus* strain URM 3371 or strain SSP 1498 suspended in 200 mL of 1 M phosphate buffer solution (Na_2HPO_4/KH_2PO_4; pH=7). The mixture was maintained at 32 °C on an orbital shaker (160 rpm) and the progress of the reaction was monitored by GC-MS as described above. After the substrate had been consumed (see Tables 1 and 2), the mixture was filtered and the aqueous phase extracted with ethyl acetate. The yellow organic phase was dried over $MgSO_4$, filtered, the solvent removed under reduced pressure and the residue purified by column chromatography over silica gel eluted with hexane and ethyl acetate mixtures (8:2 and 1:1) and with ethyl acetate to provide the secondary amines **1a** and **2a** in yields of 15–18 %.

Results and Discussion

Initially, demethylations of the benzenamines **1** and **2** were investigated using whole fungal cells of *A. terreus* strains SSP 1498, URM 3371 and URM 3571 suspended in phosphate or borate buffer solutions. The reactions were carried out using 5 g of biocatalyst and 5, 10 and 20 μL of substrates under basic (pH=9) and neutral (pH=7) conditions to ensure that the amine substrates were in deprotonated form. The results summarised in Tables 1 and 2 were the best obtained for the biotransformation of each compound after the incubation periods indicated.

Under the conditions described above, all three strains of *A. terreus* were able to efficiently biotransform *N*,*N*-dimethylbenzenamine (**1**) into *N*-methylbenzenamine (**1a**) (Table 1, entries 1, 2, 4, 5, 7 and 8) and conversion levels increased with increasing reaction time. After 10 days of incubation, the conversions were generally very high: in neutral medium, slightly higher concentrations of **1a** were observed than in alkaline buffer but the differences were not significant. The conversion levels obtained were not apparently dependent on the volume (5, 10 or 20 μL) of the added substrate, and the minor differences observed could be explained in terms of the insolubilities of the

Table 1. Demethylation of *N*,*N*-dimethylbenzenamine (**1**) by whole cells of *Aspergillus terreus*

Entry	Reaction time/day	Reaction with 5 g of cells under neutral conditions (pH=7)						Reaction with 5 g of cells under basic conditions (pH=9)					
		5 μL of **1**		10 μL of **1**		20 μL of **1**		5 μL of **1**		10 μL of **1**		20 μL of **1**	
		% **1**[a]	% **1a**[a]	% **1**[a]	% **1a**[a]	% **1**[a]	% **1a**[a]	% **1**[a]	% **1a**[a]	% **1**[a]	% **1a**[a]	% **1**[a]	% **1a**[a]
A. terreus SSP 1498													
1	5	80	20	55	45	67	33	77	23	85	15	74	26
2	10	30	70	–	100	25	65	20	80	10	90	29	71
3	15	–	–	–	–	65[b]	35[b]	–	–	–	–	–	–
A. terreus URM 3371													
4	5	41	58	67	33	81	19	45	55	55	45	85	15
5	10	–	100	–	100	58	42	40	60	28	72	48	52
6	15	–	–	–	–	65[b]	35[b]	–	–	–	–	–	–
A. terreus URM 3571													
7	5	49	51	42	58	68	32	85	15	58	42	34	66
8	10	–	100	13	87	8	73	63	37	23	77	15	85
A. terreus URM 3571[c]													
9	5	98	2	100	–	100	–	93	7	92	8	100	–
10	10	10	90	13	87	30	70	73	37	23	77	15	85

[a]in %, determined by GC-MS; [b]reaction with 15 g of cells and 100 μL of **1**; [c]whole cells stored at 4 °C for 1 year prior to the assay

Table 2. Demethylation of *N*,*N*,3-trimethylbenzenamine (**2**) by whole cells of *Aspergillus terreus*

Entry	Reaction time/day	Reaction with 5 g of cells under neutral conditions (pH=7)						Reaction with 5 g of cells under basic conditions (pH=9)					
		5 μL of **2**		10 μL of **2**		20 μL of **2**		5 μL of **2**		10 μL of **2**		20 μL of **2**	
		% **2**[a]	% **2a**[a]	% **2**[a]	% **2a**[a]	% **2**[a]	% **2a**[a]	% **2**[a]	% **2a**[a]	% **2**[a]	% **2a**[a]	% **2**[a]	% **2a**[a]
A. terreus SSP 1498													
1	5	100	–	84	16	77	23	78	22	87	13	76	24
2	10	23	76	22	78	40	60	18	82	45	55	35	65
3	15	–	–	–	–	65[b]	35[b]	–	–	–	–	–	–
A. terreus URM 3371													
4	5	47	53	60	40	74	26	53	47	58	42	72	28
5	10	–	100	42	58	40	60	24	76	40	60	35	65
6	15	–	–	–	–	65[b]	35[b]	–	–	–	–	–	–
A. terreus URM 3571													
7	5	36	76	45	55	46	56	–	–	87	13	87	13
8	10	–	100	32	68	34	66	–	100	6	94	21	79
A. terreus URM 3571[c]													
9	5	74	26	77	23	98	2	100	–	95	5	100	–
10	10	65	35	49	51	66	34	60	40	55	45	60	40

[a]in %, determined by GC-MS; [b]reaction with 15 g of cells and 100 μL of **2**; [c]whole cells stored at 4 °C for 1 year prior to the assay

substrate in the aqueous medium. Biocatalytic reactions with *A. terreus* URM 3571 were carried out using both the growing cells (resuspended in buffer solution) and the cells that had been stored at 4 °C for a period of 1 year. Although the reactions catalysed by the stored cells were slower than those observed with fresh cells during the first 5 days of incubation, *N*-demethylation occurred successfully after 10 days (Table 1, entries 9 and 10). Clearly, the enzymes that promote the demethylation of **1** are conserved in cells preserved in the refrigerator for prolonged periods of time. The *N*-demethylation reactions biocatalysed by *A. terreus* strains SSP 1498 and URM 3371 were repeated on a preparative scale using 100 μL of substrate and 15 g of whole cells (Table 1, entries 3 and 6). The conversion of **1** into **1a** was not as high as expected, probably because of the enzymatic inhibition at the high substrate concentration. Purification of the product by silica gel produced *N*-methylbenzenamine (**1a**) in only a moderate isolated yield (15–18 %).

The *N*-demethylation of *N*,*N*,3-trimethylbenzenamine (**2**) biocatalysed by whole cells of the three strains of *A. terreus* gave the desired product *N*,3-dimethylbenzenamine (**2a**) with conversion levels that were similar to those obtained for the *N*,*N*-dimethylbenzenamine **1** (Table 2). When the biotransformations of **1** and **2** by *A. terreus* URM 3571 were conducted in the presence of the oxidant *tert*-butylperoxide, the respective *N*-demethylated products **1a** and **2a** were formed at conversion levels that were not significantly different from those obtained in the absence of the additive (Table 3). However, in the presence of the oxidant, a second demethylation of **1** occurred, and both *N*-methylbezenamine (56 %) and aniline (23 %) were detected as biotransformation products. None of the studied strains of *A. terreus* were able to bring about the dealkylation of *N*,*N*-diethylbenzenamine.

According to the results presented in Tables 1 and 2, *A. terreus* strains SSP 1498, URM 3371 and URM 3571 can be considered as potential sources of enzymes for the demethylation of tertiary aromatic amines. Moreover, it is clear that benzenamines **1** and **2** represent excellent substrates for the selection of microorganisms that are capable of performing demethylation reactions.

Dealkylations of a number of amines by chemical and enzymatic methods have been previously reported (*4–11*), and peroxidases and cytochrome P450 have been involved in the *N*-demethylation of amines by haem enzymes (*12–14*). In the present study, the enzymatic demethylations promoted by *A. terreus* could occur *via*

Table 3. Demethylation of tertiary aromatic amines **1** and **2** by whole cells of *Aspergillus terreus* strain URM 3571 (5 g) under neutral conditions (pH=7)

Entry	Reaction time/day	Reaction with 20 μL of substrate **1**				Reaction with 20 μL of substrate **2**			
		with *tert*-butylperoxide (20 μL)		without *tert*-butylperoxide		with *tert*-butylperoxide (20 μL)		without *tert*-butylperoxide	
		% **1**[a]	% **1a**[a]	% **1**[a]	% **1a**[a]	% **2**[a]	% **2a**[a]	% **2**[a]	% **2a**[a]
1	4	39	61	34	66	40	60	74	26
2	8	21	56 (23[b])	26	74	33	67	44	56

[a]in %, determined by GC-MS; [b]percentage of aniline as determined by GC-MS

oxidation of the nitrogen atom by monooxygenases to form triethylamine *N*-oxide or *via* oxidative demethylation by cytochrome P450 (Scheme 1). Since these two pathways could lead to the same products, namely the *N*-demethylated compounds **1a** and **2a** according to the substrate, GC-MS/ESI analyses were carried out in an attempt to identify the possible intermediates **1b–d** and **2b–d.** Unfortunately, when *N,N*-dimethylbenzenamine (**1**) was demethylated in the presence of *A. terreus* URM 3371, no intermediates could be detected and only the product *N*-methylbenzenamine (**1a**) could be identified. It was not, therefore, possible to formulate a specific hypothesis concerning the route of demethylation of the *N,N*-dimethylbenzenamines by whole cells of *A. terreus*. Recently, the reaction of *N*-demethylation of nicotine has been investigated in the cell culture of *Nicotiana plumbaginifolia* and *N. glutinosa* using a variety of isotopic techniques (22).

Conclusions

Whole cells of the fungus *A. terreus* catalysed the *N*-demethylation of tertiary aromatic amines and, following small-scale incubation under mild conditions in aqueous medium, produced demethylated compounds at good conversions. The preliminary results described in the present study reveal that *A. terreus* offers good potential for the demethylation of tertiary aromatic amines with the additional advantage that the cells can be preserved over a long period of time without loss of activity.

Acknowledgements

The authors express their gratitude to the donors of the fungal strains from the Brazilian culture Collections of the Botanical Institute of São Paulo and the Federal University of Pernambuco. L. Piovan, V. Pellizari and L. H. Andrade thank FAPESP for fellowships. A. L. M. Porto and J. V. Comasseto thank FAPESP and CNPq (Proc. 472663/2004-6) for support.

References

1. S. Thavaneswaran, P.J. Scammells, Further investigation of the *N*-demethylation of tertiary amine alkaloids using the non-classical Polonovski reaction, *Bioorg. Med. Chem. Lett. 16* (2006) 2868–2871.
2. Z. Dong, P.J. Scammells, New methodology for the *N*-demethylation of opiate alkaloids, *J. Org. Chem. 72* (2007) 9881–9885.
3. J. Von Braun, The splitting of cyclic bases by bromocyan, *Chem. Ber. 40* (1907) 3914–3933 (in German).
4. H.G. Stenmark, A. Brazzale, Z. Ma, Biomimetic synthesis of macrolide/ketolide metabolites through a selective *N*-demethylation reaction, *J. Org. Chem. 65* (2000) 3875–3876.
5. T. Rosenau, A. Hofinger, A. Potthast, P. Kosma, A general, selective, high-yield *N*-demethylation procedure for tertiary amines by solid reagents in a convenient column chromatography-like setup, *Org. Lett. 6* (2004) 541–544.
6. S. Murahashi, T. Naota, K. Yonemura, Ruthenium-catalyzed cytochrome P-450 type oxidation of tertiary amines with alkyl hydroperoxides, *J. Am. Chem. Soc. 110* (1988) 8256–8258.
7. K. Acosta, J.W. Cessac, P.N. Rao, H.K. Kim, Oxidative demethylation of 4-substituted *N,N*-dimethylanilines with iodine and calcium oxide in the presence of methanol, *J. Chem. Soc. Chem. Commun. 17* (1994) 1985–1986.
8. E. Baciocchi, A. Lapi, Oxidation of *N*-methyl-9-*t*-butylacridane by iodosylbenzene catalyzed by tetrakis(pentafluorophenyl)porphyrin iron(III). A tool to investigate the mechanism of the oxidative *N*-demethylation of aromatic tertiary amines, *Tetrahedron Lett. 40* (1999) 5425–5428.
9. A. Pelander, I. Ojanperä, T.A. Hase, Preparation of *N*-demethylated drug metabolites for analytical purposes using 1-chloroethyl chloroformate, *Forensic Sci. Int. 85* (1997) 193–198.
10. I. Monkovic, H. Wong, C. Bachand, Secondary amines from the iron(II) ion-catalyzed reaction of amine oxides: A general method for the dealkylation of tertiary amines, *Synthesis-Stuttgart, 8* (1985) 770–773.
11. J. Santamaria, R. Ouchabane, J. Rigaudy, Electron-transfer activation. Photochemical *N*-demethylation of tertiary amines, *Tetrahedron Lett. 30* (1989) 2927–2928.
12. T.L. Macdonald, W.G. Gutheim, R.B. Martin, F.P. Guengerich, Oxidation of substituted *N,N*-dimethylanilines by cytochrome P-450: Estimation of the effective oxidation-reduction potential of cytochrome P-450, *Biochemistry, 28* (1989) 2071–2077.
13. O. Okazaki, F.P. Guengerich, Evidence for specific base catalysis in *N*-dealkylation reactions catalyzed by cytochrome P450 and chloroperoxidase. Differences in rates of deprotonation of aminium radicals as an explanation for high kinetic hydrogen isotope effects observed with peroxidases, *J. Biol. Chem. 268* (1993) 1546–1552.
14. S.B. Karki, J.P. Dinnocenzo, J.P. Jones, K.R. Korzekwa, Mechanism of oxidative amine dealkylation of substituted *N,N*--dimethylanilines by cytochrome-P-450: Application of isotope effect profiles, *J. Am. Chem. Soc. 117* (1995) 3657–3664.
15. J.I. Manchester, J.P. Dinnocenzo, L. Higgnis, J.P. Jones, A new mechanistic probe for cytochrome P450, *J. Am. Chem. Soc. 119* (1997) 5069–5070.
16. C.A. Boulton, M.J.C. Crabbe, P.J. Large, Microbial oxidation of amines. Partial purification of a trimethylamine mono-oxygenase from *Pseudomonas aminovorans* and its role in growth on trimethylamine, *Biochem. J. 140* (1974) 253–263.
17. T. Rosenau, A. Potthast, P. Kosma, C.L. Chen, J.S. Gratzl, Autocatalytic decomposition of *N*-methylmorpholine *N*-oxide induced by Mannich intermediates, *J. Org. Chem. 64* (1999) 2166–2167.
18. T. Rosenau, A. Potthast, P. Kosma, Studies into reactions of *N*-methylmorpholine-*N*-oxide (NMMO) and its hydrates with cyanuric chloride, *Tetrahedron, 58* (2002) 9809–9815.
19. J.V. Comasseto, L.F. Assis, L.H. Andrade, I.H. Schoenlein--Crusius, A.L.M. Porto, Biotransformations of *ortho*-, *meta*- and *para*-aromatic nitrocompounds by strains of *Aspergillus terreus*: Reduction of ketones and deracemization of alcohols, *J. Mol. Catal. B: Enzym. 39* (2006) 24–30.
20. C. Raminelli, E. Kagohara, V.H. Pellizari, J.V. Comasseto, L.H. Andrade, A.L.M. Porto, Biotransformations of Mannich bases and propiophenones by Brazilian microorganisms and enzymatic resolution of phenylpropanols by lipase from *Candida antarctica* (Novozym 435), *Enzyme Microb. Technol. 40* (2007) 362–369.
21. L.F. Assis, E. Kagohara, A.T. Omori, J.V. Comasseto, L.H. Andrade, A.L.M. Porto, Deracemization of (*RS*)-1-[(4-methylselanyl)phenyl]ethanol and (*RS*)-1-[(4-ethylselanyl)phenyl]-ethanol by strains of *Aspergillus terreus*, *Food Technol. Biotechnol. 45* (2007) 415–419.
22. R.J. Robins, R. Molinié, R.A. Kwiecień, P. Paneth, J. Lebreton, T.A. Bartholomeusz *et al.*, Progress in understanding *N*-demethylation of alkaloids by exploiting isotopic techniques, *Phytochem. Rev. 6* (2007) 51–63.